


## 2.2.2. Maceration Extraction (ME)

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 An abbreviated version of this protocol was published in *Pharmaceutics* in May 2021

Novel Extraction Method Using Excipients to Enhance Yield of Genistein and Daidzein in *Trifolium pratensis* L.

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### Detailed protocol

1.  $0.3 \pm 0.001$  g of dried and milled flower heads were macerated in 10 mL ethanol of selected concentration ethanol (50 or 70 % v/v).
2. After macerating samples for 12 hours, they had to be centrifuged for 10 min at 3382 g in centrifuge, followed by the decantation of the supernatant.
3. The extracts were hydrolyzed using alkaline hydrolysis - alkali hydrolysis was carried out using 25% NaOH. The samples pH was changed to 10.5 and then the extracts were sonicated at  $45 \pm 2$  °C for 10 min.
4. After hydrolysis, the sample was neutralized to pH 5.7 using 25% acetic acid.
5. After hydrolysis samples were filtered through PVDF syringe filters (pore size 0.22 µm) for further HPLC analysis.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Kazlauskaitė, J. and Bernatoniene, J. (2022). 2.2.2. Maceration Extraction (ME). Bio-protocol Preprint. [bio-protocol.org/prep1566](https://bio-protocol.org/prep1566).
2. Kazlauskaitė, J. A., Ivanauskas, L. and Bernatoniene, J. (2021). Novel Extraction Method Using Excipients to Enhance Yield of Genistein and Daidzein in *Trifolium pratensis* L.. *Pharmaceutics* 13(6). DOI: [10.3390/pharmaceutics13060777](https://doi.org/10.3390/pharmaceutics13060777)

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